

Cytokine responses and parasitic clearance in pigs infected with different dosages of *Toxoplasma gondii*

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Introduction

Toxoplasma gondii is an ubiquitous parasite of humans and domestic and wild animals. It is considered a causative agent of severe diseases in humans, with an important economic impact in domestic animals. One of the most important sources of infection in humans is the consumption of raw or undercooked meat of pigs and sheep.

Aim

The major aim of our research is to develop a vaccine for prevention of infection in pigs and sheep. Hereto, a good knowledge of the immune response following infection is essential. In the present study, we determined the cytokine responses and parasitic load in different tissues in experimentally infected pigs.

Material and methods

In the present study two groups (n=3) of 6 weeks old seronegative pigs were orally infected with a low (700 cysts) and a high (6000 cysts) dose of *T. gondii* strain *Gangji*. A third group consisted of 4 negative control animals. All animals were seronegative at the start of the experiment as determined by an indirect immunofluorescence assay (IIFA) and an IgM and IgG ELISA. Following infection, blood was collected weekly for analyzing antigen-specific serum antibody responses and monthly for isolation of PBMCs to determine antigen-specific IL-10, IL-12, IL-17 and INF- γ mRNA expression by real time quantitative PCR, upon the restimulation with 3 antigens: EC1.2, GRA7, TLA. The animals were slaughtered 4 months post infection and the following samples were collected to determine the parasitic load: brain, heart, spleen, skeletal muscles and diaphragm. The tissue fluid from the muscle samples was collected and tested in GRA7 ELISA.

Results

The responses for both IgM and IgG were comparable for the low and the high infection dose. The cytokine mRNA expression was the highest for INF- γ in all animals from both groups. The high dose group showed significant higher level of expression of INF- γ than the low dose group. The cytokine response was in all cases the strongest against TLA. The tissue fluid samples tested in all cases positive in the GRA7 ELISA, but there was no significant difference noticed between the infection doses. The parasitic load was determined by qPCR in all the samples. Surprisingly, many of the samples from both groups tested negative and the high dose group showed a stronger parasitic clearance than the low dose group.

Conclusions

This study provides the evidence that pigs infected with the low and the high dose of *T. gondii* cysts show a similar humoral response which is not protective against the infection. On the contrary, there is a significant difference in cellular response depending on the initial infection dose, resulting in different levels of INF- γ expression. Further, the results of the parasitic load suggest the appearance of parasitic clearance in several tissues, especially remarkable in the animals from the high dose group. However, further studies are required to confirm these results.

References

- Dubey J.P., Lunney J.K., Shen S.K., Kwok O.C., Ashford D.A., Thulliez P. Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. The Journal of Parasitology 1996 June; 82(3):438-43.
- Kijlstra A., Jongert E. Control of the risk of human toxoplasmosis transmitted by meat. International Journal for Parasitology 2008 October; 38(12):1359-70.
- Lind P., Haugegaard J., Wingstrand A., Henriksen S.A. The time course of the specific antibody response by various ELISAs in pigs experimentally infected with *Toxoplasma gondii*. Veterinary Parasitology 1997; 71:1-15.
- Solano A.G.I., Beshah E., Vengroski K.G., Zarlenga D., Jauregui L., Cosio M., Douglass L.W., Dubey J.P., Lunney J.K. Cytokine and lymphocyte profiles in miniature swine after oral infection with *Toxoplasma gondii* oocysts. International Journal for Parasitology 2001 February; 31(2):187-95.

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